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I.V. Kosakivska, L.V. Voytenko,
V.A. Vasjuk, M.M. Shcherbatiuk

M.G. Kholodny Institute of Botany of the NAS of Ukraine, Kyiv
E-mail: irynakosakivska@gmail.com, lesyavoytenko@gmail.com

Effect of zinc treatment on growth and phytohormones accumulation in *Triticum aestivum* L. seedlings priming with abscisic acid

Presented by Corresponding Member of the NAS of Ukraine V.V. Shvartau

We investigated whether the priming with abscisic acid (ABA) alters the growth and the content of endogenous phytohormones in winter wheat seedlings under zinc stress. It was shown that zinc at a concentration of 228 mg/l inhibits the growth of the root system. Under these conditions, a decrease in the content of endogenous indole-3-acetic acid (IAA), zeatin, and abscisic acid (ABA) and an increase in gibberellic acid (GA_3), isopentenyladenosine (iPA) and salicylic acid (SA) took place. After the adding of 10^{-6} M ABA to the incubation medium, the growth of seedling roots, the level of stressful hormones ABA and SA became higher. The strategy of the adaptation of wheat seedlings to zinc stress in the presence of exogenous ABA was aimed at the activation of root growth. Changes in the phytohormones balance initiate protective mechanisms and a further adaptation of plants to a high concentration of zinc, and the treatment of grains with exogenous ABA can be used to enhance the stress resistance.

Keywords: *Triticum aestivum L., abscisic acid, indole-3-acetic acid, gibberellic acid, salicylic acid, cytokinins, zinc.*

Heavy metals (HM), as constituents of various chemical compounds, are essential natural components of the Earth's crust. As essential microelements, they affect the plant growth, development, and metabolism. However, at levels exceeding a certain threshold, HMs act as pollutants and pose an environmental threat. Excessive concentrations of HMs inhibit the plant growth, biomass accumulation, adversely affect the photosynthetic activity, mineral nutrition, and water exchange. Zinc is one of the essential microelements necessary for the normal life of plants. Its effect is manifested in the growth promotion, initiation of flowers, formation of grains, and activation of reparative processes [1]. At high concentrations, zinc is toxic to plants. In wheat, its excess inhibited the shoot growth and reduced the rate of photosynthesis [2]. In our studies, exogenous ABA was shown to mitigate the negative effects of high zinc concentrations on the grain germination and the growth of winter wheat seedlings [3].

Plant growth and development is regulated by a complex hormonal system, the interaction between components of which is synergistic or antagonistic. Hormonal substances occur in plant tissues at low concentrations and function at sites of synthesis, or are transported to other organs and tissues. Due to their complex and balanced interaction, plants maintain hormonal homeostasis in various organs at all stages of ontogeny. In recent years, the involvement of phytohormones in the induction and integration of plant defense responses under HM impact has been actively investigated [4]. Phytohormone priming positively affects nitrogen metabolism, cell division, transpiration, and enzymatic activity under conditions of metallic stress [5]. Due to the activation of defense mechanisms and inhibition of growth and development, ABA is considered vital for the plant under the effects of biotic and abiotic stressors [6]. Therefore, our aim was to determine the effects of exogenous treatment with ABA on the accumulation and balance of phytohormones in *Triticum aestivum* L. seedlings affected by high concentrations of zinc and to investigate the possible use of the hormone to enhance the stress resistance.

Materials and methods. The seedlings of the high-yielding, frost- and drought-resistant variety *Triticum aestivum* L. Podolyanka from the collection of the Institute of Plant Physiology and Genetics of the NAS of Ukraine were studied. Dry calibrated grains were sterilized in 80 % ethyl alcohol solution, washed with distilled water, and laid out in Petri dishes (50 pcs) on filter paper moistened with distilled water (control) and filter paper moistened with solutions of zinc sulfate and 10^{-6} ABA and a mixture of zinc sulfate solutions and 10^{-6} M ABA. The plants were grown in dark at + 24 °C. The zinc solution was prepared from aqueous zinc sulfate ($ZnSO_4 \times 7H_2O$) at a content of 228 mg of pure zinc per liter.

The effect of zinc and ABA on grain germination and seedling growth was investigated on the third day of cultivation. The length and fresh weight of the most developed root, height and fresh weight of shoots were measured. Biological replication within each variant of the experiment was 10 plants in three times analytical replication.

Phytohormones were isolated and subsequently purified from 2 g of plant material according to the method [7] on two solid-phase columns SPE C18, Sep-Pak Plus, Waters and SPE Oasis MCX, 6 cc / 150 mg, Waters. Analytical determination of phytohormones was performed using high performance liquid chromatography on an Agilent 1200 LC liquid chromatograph with a G 1315 V diode-matrix detector (USA) in tandem with an Agilent G6120A single-quadrupole mass spectrometer. To obtain the calibration tables, unlabelled IAA, ABA, CK, GA_3, *trans*-zeatinglucoside (*t*-ZG), *trans*-zeatin (*t*-Z), *trans*-zeatinriboside (*t*-ZR), isopentenyladenine (iP), and isopentenyladenosine (iPA) manufactured by Sigma-Aldrich, USA were used. The experiments were carried out in three biological and three analytical replicates. The content of phytohormones was analyzed and calculated using Agilent OpenLAB CDS ChemStation Edition software (rev. C.01.09). The quantitative values obtained were statistically processed using Microsoft Excel 2016. The significance of the difference was estimated according to Student's criterion using a 5 % significance level ($P \leqslant 0.05$, 0.01 and 0.001).

Results and discussion. ***Morphological studies.*** The seed germination, root system formation, and tillering are the decisive stages of ontogenesis, which affect the productivity of crops and wheat yield. Morphometrical parameters of growth under stress factors are integral characteristics of the plant physiological state. On the first day of seed germination, enzymes are activated; the intensity of respiration increases, hydrolysis of the reserve substances used by the embryo for germination takes place. On the 2-3rd day, embryo roots emerge, which provide the inflow of moisture and minerals to the seedling. Under favorable conditions when germi-

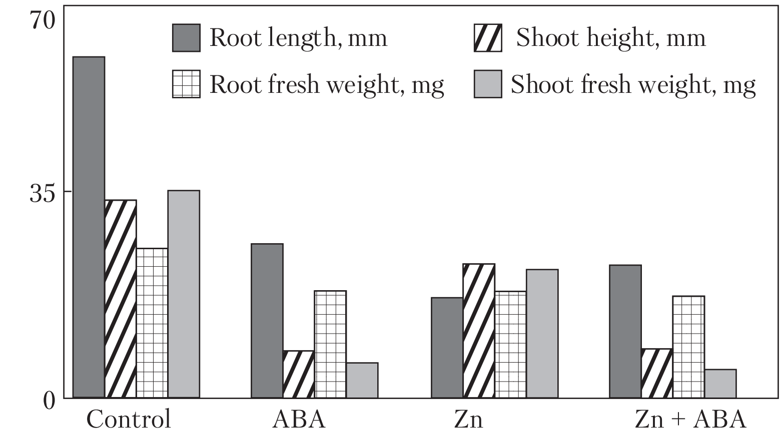

Fig. 1. Morphometrical study of three-day-old *Triticum aestivum* L. cv. Podolyanka seedlings grown on ABA and zinc solutions

nating in the soil for 3-4 days, the shoot goes to the surface. During this period, seedlings are going from heterotrophic to autotrophic nutrition.

In the model we created, all solutions inhibited the growth of seedlings. Exogenous ABA slowed shoot development, and zinc sulfate reduced root growth (Fig. 1).

Their combined application mitigated the individual inhibitory effects of these substances, but growth rates remained to be less than those of controls. It should be noted that the biomass of seedlings grown on a zinc sulfate solution was higher. Analysis of the biomass of seedlings indicated that it increase was due to shoots development. When incubated with ABA+zinc, seedlings biomass was less than control (see Fig. 1).

Phytohormones accumulation. *Auxins* are involved in the regulation of the seed germination, division, elongation and differentiation of cells, photo- and gravitropisms, apical dominance, embryo-, organo- and morphogenesis, development of the root system [8]. As a result of the HM effect, auxin homeostasis is disturbed, and that adversely affects plant growth and development. Zinc has been reported to be involved in IAA metabolism, and the excess metal influences the hormone content [1]. We showed that incubation on ABA and zinc sulfate solutions caused, respectively, a two-fold and three-fold fall in endogenous IAA content in three-day-old wheat seedlings, whereas after the combined use of phytohormone and HM – 2.6 times. Thus, the addition of the hormone slightly mitigated the negative effect of zinc on the accumulation of IAA (Table).

Abscisic acid plays a key role in the formation of plant defense mechanisms, regulation of ripening and seed germination, and influences the architecture of the root system [6].

Phytohormones content in three-day-old *Triticum aestivum* L. cv. Podolyanka seedlings grown on ABA and zinc solutions

Study variant	IAA	Cytokinins		GA	ABA	SA
		t-Z	iPa			
Control	92.3 ± 4.6	4.3 ± 0.2	1.0 ± 0.05	7.4 ± 0.4	10.9 ± 0.5	1.4 ± 0.1
10^{-6}M ABA	28.2 ± 1.4	12.2 ± 0.6	0.7 ± 0.04	1.7 ± 0.1	114.9 ± 5.7	9.9 ± 0.5
Zn 228 mg/l	47.3 ± 2.2	3.2 ± 0.2	1.3 ± 0.06	10.0 ± 0.5	9.9 ± 0.5	2.9 ± 0.1
Zn 228 mg/l + 10^{-6}M ABA	34.8 ± 1.7	2.9 ± 0.1	0.6 ± 0.03	1.9 ± 0.1	38.2 ± 1.9	11.6 ± 0.6

The decrease of ABA content in the roots and a rise in poplar leaves was observed after a prolongation of the zinc stress under conditions of seedlings exposure to exogenous ABA [9]. However, zinc was reported not to affect or reduce the hormone content in Arabidopsis shoots [10]. Our study showed that, when grown on ABA solution, the content of endogenous ABA in three-day-old winter wheat seedlings increased 10.5 times. During the action of zinc, it remained at the control level, whereas the combined use of ABA and zinc caused a 4-fold rise in the hormone content. Incubation with ABA solution mitigated the negative effect of zinc on wheat root growth (see Table, Fig. 1).

Gibberellins are activators of processes that occur during the seed germination, they stimulate linear growth of axial organs and increase the leaf surface [11]. Gibberellins content in plant tissues is significantly affected by environmental conditions. Thus, the high concentration of zinc caused a fall in GA_3 content in the poplar roots and leaves irrespective of the exposure duration [9]. When the zinc concentration was low, there was observed accumulation of endogenous GA_3 in the germinating seeds of *Cicer arietinum*, while the hormone content decreased at its high concentration. However, all studied concentrations of HMs caused a significant delay in the seed germination [12]. We demonstrated that incubation on zinc solution enlarged the content of endogenous GA_3 in wheat seedlings, whereas the amount of hormone decreased almost fourfold when there were treated by ABA solutions and a mixture of ABA and zinc sulfate (see Table). Zinc had a positive effect on the accumulation of GA_3, but an enlargement of the three-day-old wheat seedlings shoots was not observed (see Fig. 1).

Cytokinins includes adenine derivatives; the compounds are similar in structure, but different in biological activity and functions. Hormone molecules with some variations in the structure of the side chain are likely to mediate different biological signals: it has been established that *trans*-zeatin and iPa are involved in the transmission of long-range signals in the acropetal and basipetal directions, respectively. Cytokinins control the cell division, stimulate the formation and activity of shoot meristems, form the tissue attractive capacity, repress the leaf senescence, inhibit root growth and branching, and are involved in regulating the seed germination and response to stress [13]. During the germination of chickpea seeds under zinc stress, a gradual increase in the zeatin content and a decrease in the zeatin riboside one were detected. Zeatin is suggested to play a dominant role in overcoming such stress and can be synthesized *de novo* [12]. In our study, the cytokinins zeatin-*O*-glucoside, zeatin riboside, and isopentenyladenine were found in trace amounts (less than 0.5 ng/g of crude substance) in three-day-old winter wheat seedlings. Only zeatin and iPA were identified in significant quantities. Exogenous ABA caused a threefold rise in the zeatin content, whereas zinc partially suppressed the hormone accumulation, and the lowest level of zeatin was recorded under the combined action of exogenous ABA and HM. The iPa accumulation pattern had the opposite direction, namely, as a result of the exogenous ABA effect, the hormonal content diminished while the zinc effect led to its increase. Growing on a mixture of ABA and HM inhibited iPa accumulation (see Table).

Salicylic acid plays a key role in the formation of the resistance of germinating seeds and adult plants under various biotic stressors, and thus, the hormone is positioned as an effective remedy [14]. HM action was reported to enhance the exogenous SA antioxidant protection, to mitigate lipid peroxidation, and to increase photosynthetic activity [5]. Some rise in SA biosynthesis during HM impact in roots and poplar leaves was observed [9]. We established that,

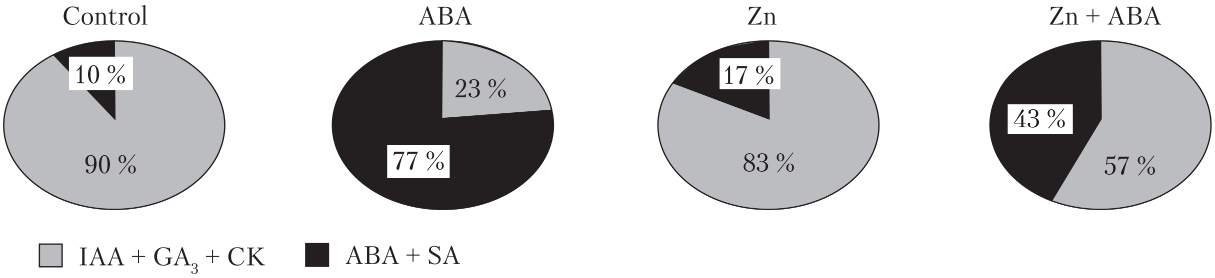

Fig. 2. Ratio between IAA+GA_3+CK content and ABA+SA content in three-day-old *Triticum aestivum* L. cv. Podolyanka seedlings grown on ABA and zinc solutions (%)

when incubated on a solution of zinc sulfate, three-day-old wheat seedlings showed a two-fold increase in SA content, and it increased seven times with exogenous ABA. A ten-fold rise in SA content was observed with the combined action of zinc sulfate and ABA solutions (see Table).

Endogenous phytohormones ration. Under the control growing conditions, endogenous IAA in three-day-old seedlings of winter wheat dominated, its content reached 92 ng/g of fresh weight. As a result of the zinc effect, IAA also dominated. However, the content of IAA was twice below the control. With the combined action of zinc and ABA, the content of IAA and ABA was close to 34.8 ± 1.7 and 38.2 ± 1.9 ng/g of fresh substance, respectively. Under the same conditions, the content of SA was maximal. The ratio of total content of hormones stimulator IAA, GA_3, and CK to total contents of stress hormones ABA and SA under control conditions was 9:1, for ABA actions — 0.3 : 1, zinc — 4.8 : 1, zinc + ABA — 1.3 : 1 (Fig. 2). The results obtained indicate that the grains priming with a solution of ABA initiates the accumulation of stress hormones that are a trigger in the formation of stress resistance of wheat plants in the early stages of their seedlings development.

The pattern of changes in the endogenous phytohormones content showed that zinc and exogenous ABA acted as abiotic stressors. Changes in morphometric parameters observed in our study indirectly indicate the involvement of stimulating hormones IAA, Z, iPA, and GA_3 in the regulation of shoots growth under zinc stress. Some rise in SA content occurred in all experimental variants, testifying the hormone involvement to the formation of defense mechanisms.

Our study showed that high zinc concentrations caused the changes in hormones accumulation and balance in winter wheat seedlings at the juvenile stage of their development. Zinc at a concentration of 228 mg/l inhibited the growth of the root, which was the first to be adversely affected by the excessive concentration of HM. Under these conditions, a fall in endogenous IAA, zeatin, and ABA contents and a rise in GA_3, iPA, and SA were observed. Seedlings incubation on 10^{-6} M ABA solution induced the growth of the root system. A combined action of the hormone and zinc resulted in the leveling of the HM inhibitory effect, and the content of stress hormones SA and ABA increased. Our results are consistent with those of other researchers regarding the involvement of ABA in triggering SA and IAA signaling cascades capable of reducing the toxic effect of HM [15]. Therefore, the strategy of wheat seedlings adaptation to zinc loading under the action of exogenous ABA was aimed at inhibiting the shoot growth and activating the root growth. Changes in the balance of phytohormones are able to initiate protective mechanisms and a further adaptation of plants to the effects of excessive HM concentrations, and the priming of grains with exogenous ABA can be used to improve the stress resistance.

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І.В. Косаківська, Л.В. Войтенко,
В.А. Васюк, М.М. Щербатюк

Інститут ботаніки ім. М.Г. Холодного НАН України, Київ
E-mail: irynakosakivska@gmail.com, lesyavoytenko@gmail.com

ВПЛИВ ЦИНКУ НА РІСТ І АКУМУЛЯЦІЮ ФІТОГОРМОНІВ ПРОРОСТКАМИ *TRITICUM AESTIVUM* L., ПРАЙМОВАНИМИ АБСЦИЗОВОЮ КИСЛОТОЮ

Досліджено вплив цинкового стресу на ріст та акумуляцію ендогенних фітогормонів у проростках озимої пшениці, зернівки якої були праймовані абсцизовою кислотою (АБК). Показано, що в результаті інкубації на розчині цинку надлишкової концентрації (228 мг/л) гальмувався ріст кореневої системи, знижувався вміст ендогенної індоліл-3-оцтової, абсцизової кислот і зеатину та збільшувалась кількість гіберелінів, ізопентеніладенозину та саліцилової кислоти. Після додавання в інкубаційне середовище 10^{-6} М АБК посилювався ріст коренів, вміст стресових гормонів абсцизової та саліцилової кислот зростав. Зміни у балансі фітогормонів ініціювали захисні механізми рослин до дії високої концентрації цинку, а отже, праймування зернівок екзогенною АБК може бути використане для підвищення стресостійкості.

Ключові слова: *Triticum aestivum L.*, абсцизова кислота, індоліл-3-оцтова кислота, гібереліни, саліцилова кислота, цитокініни, цинк.

И.В. Косаковская, Л.В. Войтенко,
В.А. Васюк, Н.Н. Щербатюк

Институт ботаники им. Н.Г. Холодного НАН Украины, Киев
E-mail: irynakosakivska@gmail.com, lesyavoytenko@gmail.com

ВЛИЯНИЕ ЦИНКА НА РОСТ И АККУМУЛЯЦИЮ ФИТОГОРМОНОВ ПРОРОСТКАМИ *TRITICUM AESTIVUM* L., ПРАЙМИРОВАНЫМИ АБСЦИЗОВОЙ КИСЛОТОЙ

Исследовано влияние цинкового стресса на рост и аккумуляцию эндогенных фитогормонов проростками озимой пшеницы, зерновки которой были праймированы абсцизовой кислотой (АБК). Показано, что при инкубации на растворе цинка избыточной концентрации (228 мг/л) угнетался рост корней, уменьшалось количество эндогенных индолил-3-уксусной, абсцизовой кислот, зеатина и увеличивалось содержание гиббереллинов, изопентениладенозина и салициловой кислоты. После добавления в инкубационную среду 10^{-6} М АБК рост корней усиливался, содержание стрессовых гормонов абсцизовой и салициловой кислот возрастало. Изменения в балансе фитогормонов инициировали защитные механизмы растений к действию высоких концентраций цинка, следовательно, праймирование зерновок экзогенной АБК может быть использовано для повышения стрессоустойчивости.

Ключевые слова: *Triticum aestivum L., абсцизовая кислота, индолил-3 уксусная кислота, гиббереллины, салициловая кислота, цитокинины, цинк.*